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SHORT COMMUNICATION

Mutational Analysis of the Conserved Cysteine Residues in the Simian Immunodeficiency Virus Matrix Protein

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The matrix protein (MA) of human and simian immunodeficiency viruses (HIV and SIV) is encoded by the amino-terminal region of the Gag precursor and has been suggested to be involved in different processes during the early and late stages of the virus life cycle. The MA protein of SIV contains three cysteine residues at positions 57, 83, and 87, which are also highly conserved among HIV-2 isolates. In order to study the functional significance of these residues in virus morphogenesis, a series of mutations affecting the cysteines of SIV MA were introduced into a *gag*–protease construct and expressed in the vaccinia vector system. The MA mutants were assayed for their ability to synthesize and process the Gag polyprotein precursor as well as to release particles into the culture medium. In addition, the incorporation of the envelope glycoprotein (Env) into the Gag-made particles was investigated. Substitution of alanine for cysteine 87 had little effect on particle release and Env glycoprotein association. By contrast, the individual replacement of cysteines 57 or 83 by alanine, as well as the simultaneous mutation of cysteines 83 and 87, significantly reduced the ability of Gag polypeptides to produce extracellular particles. Assembly into particles appeared to be also affected, albeit to a lesser extent, when both cysteines 57 and 83 were replaced by alanine. Furthermore, substitution of cysteine 83 in the SIV MA domain was found to be detrimental to Gag polyprotein processing. Analysis of the Env glycoprotein association with recombinant particles revealed that this process was moderately affected in the case of the double mutants lacking cysteines 57 and 83, or cysteines 57 and 87, and the cysteine-minus triple mutant. Our results suggest that the conserved cysteines 57 and 83 in the MA domain are important for efficient SIV Gag particle production.

In the late stages of replication, human immunodeficiency viruses (HIV) and the closely related simian immunodeficiency viruses (SIV) assemble their capsid shell from the viral Gag polyproteins, and the particle then buds from the plasma membrane of the infected cell. The Gag proteins are synthesized as a polyprotein precursor ($Pr55^{Gag}$) which is proteolytically processed by the viral protease into the mature Gag proteins matrix (MA or p17), capsid (CA or p24), nucleocapsid (NC or p7), and p6 (*1, 2*). The HIV and SIV MA protein forms the outer shell of the core of the virus, lining the inner surface of the viral membrane (*3*). As with most mammalian retroviruses, the HIV and SIV N-terminal MA domain is cotranslationally modified by addition of myristic acid, which serves as a membrane targeting signal (*4–6*). In addition to myristylation, a polybasic region within the NH_2-terminus of MA appears to be necessary for proper membrane targeting of the $Pr55^{Gag}$, as has been demonstrated for HIV-1 (*7–10*) and SIV (*11*).

Several functions have been proposed for the HIV and SIV MA protein. At early steps in the virus cycle, HIV-1 MA may be involved in virus entry (*12*), nuclear localization of the preintegration complex after virus entry (*13*), and RNA binding (*14*). At late stages of the viral cycle, the MA plays a role in virion morphogenesis by being involved in the incorporation of the viral envelope (Env) glycoprotein into HIV-1 (*15, 16*) and SIV particles (*11*). Furthermore, mutational analyses within the MA domain of HIV-1 (*15, 17, 18*), together with the effect on HIV-1 morphogenesis of competing synthetic peptides derived from the MA sequence (*19*), have suggested that the MA protein may also be important for virus particle assembly. However, it has been reported that large deletions of the HIV-1 MA appear to have no effect on particle production (*8, 20*). In SIV, we have shown that the MA protein, when expressed alone in the absence of other viral components, assembles into lentivirus-like particles which are released into the culture medium and incorporate the viral Env glycoprotein (*11*).

Besides the N-myristylation signal (*21*) and the membrane-targeting polybasic region (*11*), no other domains responsible for SIV MA function have been identified so far. The MA domain of SIV_{smmPBj} Gag polyprotein contains three cysteine residues at positions 57, 83, and 87, which are highly conserved among SIV and HIV-2 isolates (*22*) (Fig. 1). Interestingly, C57 and C87 are also present in

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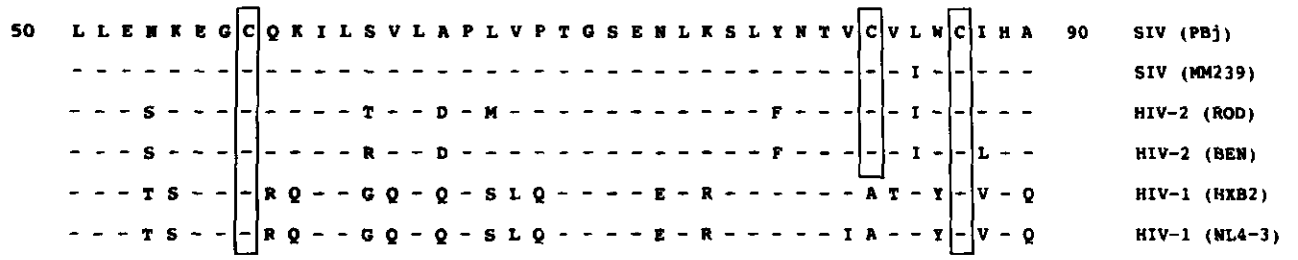

FIG. 1. Sequence alignment of the region representing residues 50 to 90 of the MA domain of SIV and HIV isolates. The highly conserved cysteine residues among the sequences are highlighted by an open box. Dashes within the sequences indicate identical amino acids to those of SIV_{smmPBj}.

the MA protein of diverse HIV-1 isolates (Fig. 1). The aim of the present study was to determine the function and significance of the conserved SIV MA cysteines during virus morphogenesis. To this end, we generated a series of MA mutants in which one, two, or all cysteine residues were substituted with alanine. This amino acid was chosen as the replacement residue because it eliminates the side chain beyond the β-carbon yet does not alter the main-chain conformation. Furthermore, alanine is frequently found in both buried and exposed positions and in a variety of secondary structures (*23*). The codons for the cysteine residues of SIV MA were changed to codons specifying alanine in a construct encompassing the complete *gag* gene in addition to the *pol* region coding for the protease domain (Gag–PR) (Fig. 2A). Changes in the coding sequence of the SIV_{smmPBj} MA were introduced by asymmetric PCR-based site-directed mutagenesis as described before (*11*). Mutagenesis was performed on a *SalI/PstI* restriction fragment (nt 828–1646) cloned into the pUC19 vector which corresponds to the first 138 amino acids of SIV Gag polyprotein. The SIV MA cysteine residues at positions 57, 83, and 87 (referred to as C57, C83, and C87, respectively) were individually converted to alanine with the following antisense mutagenic oligonucleotides, respectively: C57A, 5′-TTTTTGAGCACC-TTCTTTGTTC-3′ (nt 1004–983); C83A, 5′-GCACCAAAG-GACGGCGACAGTG-3′ (nt 1088–1067); C87A, 5′-TCT-GCGTGAATGGCCCAAAGGA-3′ (nt 1099–1078). For designation of each mutation, the wild-type (wt) amino acid is indicated, and then its position and the mutant amino acid are listed. In the DNA sequences the corresponding antisense alanine codons are underlined. The

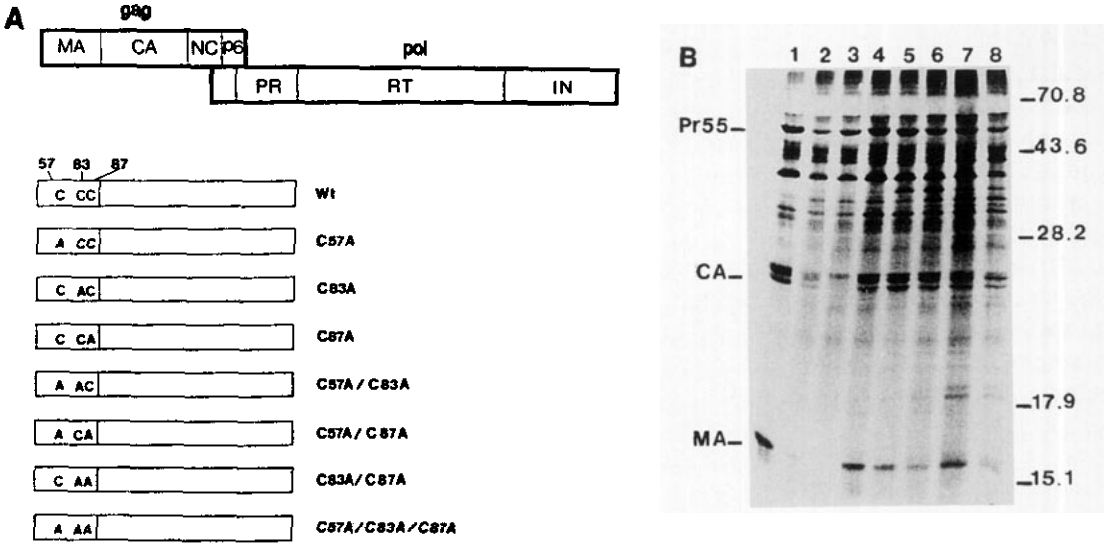

FIG. 2. Construction and expression of SIV *gag* vaccinia recombinants. (A) Schematic diagram of wild-type and mutant SIV Gag–PR constructs. At the top of the figure, the *gag–pol* open reading frames of SIV and the proteins they encode are indicated as open boxes (PR, protease; RT, reverse transcriptase; IN, integrase). In the lower part of the figure, the Gag–PR constructs in which the conserved MA cysteine residues were replaced by alanine are shown. (B) Expression of mutant SIV Gag proteins by recombinant vaccinia viruses. CV-1 cells (4×10^5) were infected at high multiplicity (10 PFU per cell) in parallel with vv-wtgag (lane 1) or *gag* mutants (lanes 2–8). At 4–5 hr postinfection cell cultures were metabolically labeled for 3–4 hr with [^{35}S]methionine (100 μCi/ml; >1000 Ci/mmol; Dupont NEN) in methionine-deficient DMEM (GIBCO BRL) supplemented with 2% fetal bovine serum. Cells were washed with cold phosphate-buffered saline (PBS) and the cells were lysed at 4° in 100 μl TNN lysis buffer [50 m*M* Tris–HCl (pH 8.0), 150 m*M* NaCl, 1% NP-40, 1 m*M* phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin]. Lysates were spun at 12,000 *g* for 2 min and the postnuclear supernatants were subjected to immunoprecipitation with pooled sera from SIV-infected macaques, followed by fractionation on a SDS–15% polyacrylamide gel. Immunoprecipitations were carried out as previously described (*34*). The position of virus-encoded proteins are shown on the left. Numbers on the right correspond to the position of molecular weight standards (in kilodaltons).

C57A/C83A and the C57A/C87A double mutants were created by mutating C83 and C87, respectively, on the C57A parent. The C83A/C87A double mutant was generated using the following antisense oligonucleotide: 5′-GAATGGCCCAAAGGACGGCGACAGTG-3′ (nt 1092–1067). The C57A/C83A/C87A triple mutant was created by simultaneous mutagenesis of C83 and C87 using the C57A DNA as template. Double-stranded DNA sequencing was carried out for each mutant to confirm the presence of the corresponding mutations and the absence of nucleotide misincorporations due to the gene amplification step. The mutated *SalI/PstI* fragments were substituted for the wt counterpart in the pMJ601 vaccinia transfer vector containing the SIV wt *gag* and PR genes (*11*). The mutated *gag* sequences were inserted into the vaccinia WR virus by homologous recombination between the plasmid transfer vector pMJ601 and vaccinia virus genomic DNA essentially as described (*11*). Selection and purification of recombinant vaccinia viruses were performed as described previously (*11*). At least two independent recombinant vaccinia viruses of each *gag* mutant were initially screened to be sure that their *gag* products exhibited the same phenotype.

To examine the synthesis and processing of mutated SIV Gag polypeptides, CV-1 cells were infected with the recombinant vaccinia viruses and metabolically labeled with [^{35}S]methionine. The Gag-specific polypeptides were immunoprecipitated from cell lysates with pooled sera from SIV-infected macaques (Fig. 2B). Cells infected with the recombinant vaccinia virus expressing wild-type Gag–PR (vv-wtgag), as well as with the MA mutants, expressed the Pr55^{Gag} precursor protein, which was partially processed to the mature CA and MA proteins. The sole exception was mutant C83A, in which the CA and MA proteins were not detected (Fig. 2B, lane 3). This pattern suggested that this mutant might be defective in polyprotein processing. The possibility of an error in the Gag- and PR-coding sequences of mutant C83A was ruled out by DNA sequencing. All these polypeptides are specific to SIV since none of them were detected in cells infected with a vaccinia virus expressing only β-gal used as negative control (data not shown). In addition to the Pr55^{Gag} precursor and final products CA and MA, a Gag-related polypeptide of 39–40 kDa was detected by immunoprecipitation of all cell lysates. This polypeptide may represent either a Gag-processing intermediate (encompassing the MA–CA or CA–NC domains) generated by the viral PR (*24*) or, alternatively, the proteolytic product of host-encoded proteases. Indeed, a 39- to 41-kDa band is detected when the Pr55^{Gag} polyprotein of HIV and SIV is expressed by recombinant vaccinia and baculoviruses lacking PR function (*21, 25*).

To study the contribution of the conserved cysteine residues in the MA domain to the assembly process of SIV, the MA mutants were tested for their ability to form and release particles into the culture medium. In addition,

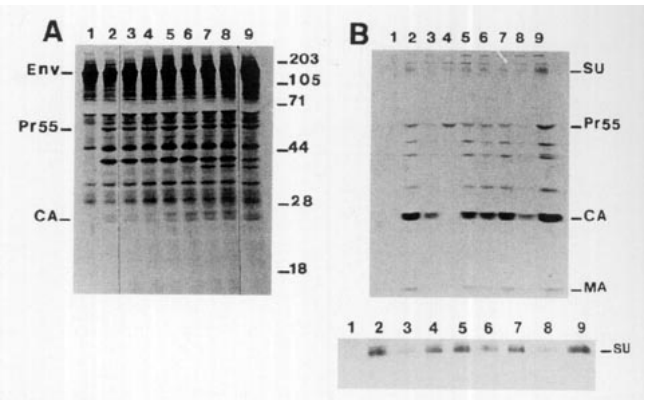

FIG. 3. Effect of MA mutations on particle production and viral Env incorporation. (A) Analysis of cell lysates. CV-1 cells were infected with vv-env alone (10 PFU per cell), or coinfected with vv-env and either vv-wtgag or an MA mutant (5 PFU per cell of each virus). Cells were metabolically labeled with [^{35}S]methionine and cell lysates were analyzed by immunoprecipitation and SDS–10% polycrylamide gel electrophoresis. The respective positions of SIV-specific proteins are noted. (B) Analysis of the particulate fraction. Medium from infected cultures was collected at 8–9 hr postinfection, clarified three times by centrifugation at 800 *g* for 10 min, and then overlaid onto a 20% (w/v) sucrose cushion in PBS buffer. Samples were centrifuged at 120,000 *g* for 90 min in a Beckman SW50.1 rotor at 4°. The pellet (corresponding to particulate material) was resuspended in TNN lysis buffer and assayed for SIV proteins by immunoprecipitation. (Bottom) Enlargement of a longer exposure of the region of the autoradiogram corresponding to the SU protein. Lane 1, lysate (A) and pellet (B) fractions from cells infected with vv-env alone. Lanes 2–9, lysate (A) and pellet (B) fractions from cells coinfected with vv-env and vv-wtgag (lane 2), or C57A (lane 3), C83A (lane 4), C87A (lane 5), C57A/C83A (lane 6), C57A/C87A (lane 7), C83A/C87A (lane 8), C57A/C83A/C87A (lane 9).

the effect of MA mutations on Env glycoprotein incorporation into particles was investigated in the same experiment. To this end, cells were coinfected with each MA mutant and vv-env, a vaccinia recombinant expressing SIV$_{smmPBj}$ Env glycoproteins (*26*), and metabolically labeled with [^{35}S]methionine for 3 hr. Cell lysates were assayed for the presence of SIV Gag and Env proteins by immunoprecipitation. The particulate material released into the culture supernatant was pelleted through a 20% sucrose cushion and analyzed likewise. As shown in Fig. 3A, coinfection experiments yielded similar levels of Env glycoproteins and Gag polypeptides in cell lysates. We have previously shown by both biochemical and electron microscopic analyses that coinfection with vv-wtgag and vv-env results in extracellular release of virus-like particles carrying the viral Env glycoprotein (*11, 26*). When particle production of MA mutants was compared to that of wild-type Gag, their phenotypes could be arranged into three groups (Fig. 3B). Single mutant C87A, double mutants C57A/C83A and C57A/C87A, and the triple mutant C57A/C83A/C87A produced particles with efficiency similar to, or in the case of the triple mutant, greater than that of wild-type Gag. In contrast, single mutant C57A and double mutant C83A/C87A yielded significantly lower levels of Gag particulate material. The

TABLE 1

Particle Release and Env Protein Incorporation into Particles[a]

Construct	% Particle production[b]	Env association index[c]
Wild-type gag	100	1.0
C57A	31	1.1
C83A	28	2.3
C87A	78	0.9
C57A/C83A	55	0.6
C57A/C87A	96	0.5
C83A/C87A	34	1.0
C57A/C83A/C87A	180	0.6

[a] CV-1 cells were coinfected with vv-env and vv-wtgag or the MA mutants. Virus-like particles from 0.5 ml culture medium of infected cells were sedimented by ultracentrifugation through a 20% sucrose cushion followed by immunoprecipitation with pooled sera from SIV-infected macaques and analysis by SDS–PAGE. Bands corresponding to $Pr55^{Gag}$, CA, and SU proteins were excised from the dried gel after fluorography and their radioactivity was measured in a liquid scintillation counter. Values represent the means of three independent experiments. Standard deviations were in all cases less than 15%.

[b] The percentages were obtained by considering the radioactivity of the $Pr55^{Gag}$ plus CA bands in the pellet fraction from vv-wtgag-infected cells to be 100%.

[c] The Env-particle association index was calculated by using the following formula: $[(SU)_{mutant} \times (Pr55^{Gag} + CA)_{wild\text{-}type}]/[(Pr55^{Gag} + CA)_{mutant} \times (SU)_{wild\text{-}type}]$.

third pattern corresponds to the C83A mutation which resulted in inefficient release of unprocessed $Pr55^{Gag}$. The amount of Gag-related polypeptides in the particulate material released by each MA mutant was quantitated and compared with that of wild-type Gag. The results obtained from three independent experiments are shown in Table 1. The level of particulate Gag in the culture medium of cells expressing the C57A and C83A/C87A MA mutants was reduced by about 70%, suggesting that these mutations may cause a major defect in particle release. The substitution of C83 with alanine in the SIV MA, which impaired Gag polyprotein processing (Fig. 2B), also reduced particle release by 72%. Replacement of both C57 and C83 by alanine in SIV MA caused a 45% reduction in the amount of particulate Gag proteins. Substitution of all three cysteine residues (mutant C57A/C83A/C87A) resulted in an almost twofold increase in particle release, whereas mutants C87A and C57A/C87A yielded amounts of particle-associated Gag proteins which were similar to those obtained with the wild-type Gag construct. It should be pointed out that when the MA mutants were expressed alone in the absence of the Env glycoproteins, the same pattern of particle production was observed (data not shown).

To study the effect of the MA mutations on the Env glycoprotein association with Gag particles, we assayed the particulate fraction of culture supernatants for the presence of the surface protein gp120 (SU). In the pellets of wild-type Gag particles, significant amounts of SU protein were detected (Fig. 3B, lane 2). When mutant particles were analyzed, different amounts of SU protein were detected above background levels (Fig. 3B, lanes 3–9). The presence of the SU protein in the particulate fraction could only be attributed to specific interactions between Gag and Env proteins since SIV Env glycoproteins, when expressed alone by the vv-env vaccinia recombinant, were not detected in the pelleted material from cell-culture supernatants (Fig. 3B, lane 1).

An Env-particle association index was calculated as an estimate of the efficiency of association of SU protein with mutant Gag particles relative to that with wild-type Gag particles (Table 1). Mutants C57A, C87A, and C83A/C87A showed an Env-particle association index which was similar to that of wild-type Gag. In contrast, mutants C57A/C83A, C57A/C87A, and C57A/C83A/C87A exhibited an index of 0.5–0.6, which indicates that mutant particles incorporated 40–50% less SU protein than did wild-type Gag particles. Interestingly, substitution of C83 with alanine increased the ability of mutant particles to associate with the Env glycoprotein, as evidenced by a higher association index with respect to that of wild-type Gag. The observed differences in SU protein incorporation into particles among MA mutants were not due to significant variation in the intracellular levels of Env glycoproteins in coinfected cells (Fig. 3A). Since mutation of the conserved cysteine residues in the SIV MA resulted in different phenotypes with regard to particle release, we studied the kinetics of Gag polyprotein processing and release into the culture medium as particulate material. To this end, pulse–chase experiments were performed on MA mutants representing each phenotype. Cells expressing each MA mutant were pulse-labeled for 20 min or pulse-labeled and then chased in complete medium for different lengths of time. Both cell-associated and released Gag polypeptides were analyzed by immunoprecipitation followed by SDS gel electrophoresis (Figs. 4 and 5). When cells infected with vv-wtgag were pulse-labeled for 20 min, the band of viral $Pr55^{Gag}$ was detected (Fig. 4A, wt, lane P). The processing of wild-type $Pr55^{Gag}$ polypeptide was evidenced after a 1.5-hr chase by the decrease in the intensity of the precursor band and the appearance of the final CA and MA products (lane 1). After a 4.5-hr chase, most of the intracellular Gag precursor appeared to be processed (lane 2). After a 1.5-hr chase, Gag was detected as particle-associated proteins in the culture medium (Fig. 4B, wt, lane 1). Consistent with decreasing amounts of cell-associated CA and MA proteins, increasing levels of extracellular CA and MA proteins were observed following the 4.5-hr chase (Fig. 4B, wt, lane 2). Mutant C87A was chosen to illustrate the Gag polyprotein processing of those MA mutants showing a particle production phenotype similar to that of wild-type Gag. In cells infected with mutant C87A, Gag polyproteins were processed to mature proteins and released as particulate material with

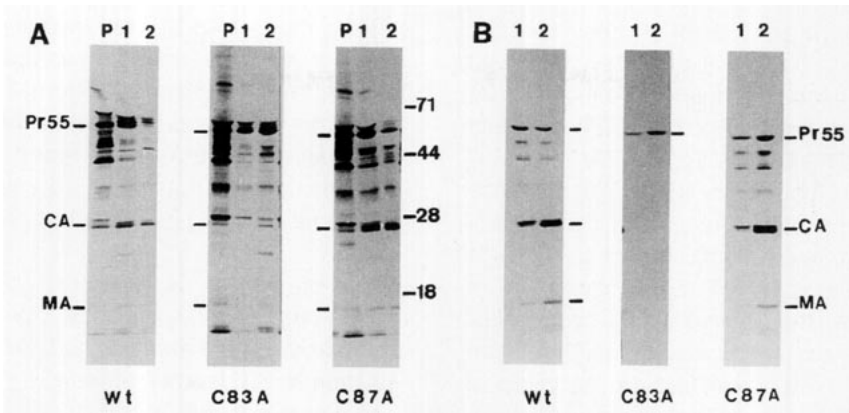

FIG. 4. Kinetics of Gag polyprotein processing and particle release of mutants C83A and C87A. (A) Analysis of cell-associated viral proteins. CV-1 cells infected with vv-wtgag (wt) or MA mutants were pulse-labeled (lane P) with [^{35}S]methionine (250 μCi/ml) for 20 min and chased in complete DMEM for 1.5 hr (lane 1) and 4.5 hr (lane 2). Cells were lysed and viral proteins were immunoprecipitated with pooled sera from SIV-infected macaques. (B) Analysis of viral proteins in the particulate fraction of the culture supernatants. The culture media of cells infected with vv-wtgag or the MA mutants were harvested after 1.5-hr (lane 1) and 4.5-hr (lane 2) chases. The particulate material was pelleted through a 20% sucrose cushion and assayed for SIV proteins by immunoprecipitation.

a slightly slower kinetics than that of wild-type Gag (Figs. 4A and 4B, C87A, lanes P–2). A different pattern was observed for the mutant C83A. The C83A Gag precursor was synthesized at a normal level (Fig. 4A, C83A, lane P), and it remained unprocessed after a 4.5-hr chase (Fig. 4A, C83A, lane 2), although some turnover of the precursor was observed over time. Only a faint band corresponding to CA was detected, which evidenced a very low level of processing (Fig. 4A, C83A, lane 2). Analysis of the particulate fraction obtained following the chase periods showed that mutant C83A particles consisted of unprocessed precursor (Fig. 4B, lanes 1–2). We therefore conclude that mutation of C83 in the SIV MA has a drastic effect on Gag polyprotein processing.

Figure 5 shows the kinetics of Gag polyprotein processing and particle release of mutant C57A which, in the experiments of particle production described above, was found to release particles less efficiently than the wild-type construct (Fig. 3B). After a 20-min pulse, cells infected with mutant C57A synthesized the Gag polyprotein precursor at levels comparable to those of vv-wtgag-infected cells (Fig. 5A, lanes P). Following a 1.5-hr chase, the mutant Gag precursor appeared to be cleaved into the mature CA and MA proteins; similar levels of intracellular CA protein were evident in both mutant C57A- and vv-wtgag-infected cells (Fig. 5A, lanes 1). However, the CA protein was barely detectable in the particulate fraction of mutant C57A at this time point (Fig. 5B, lanes 1). After a 4-hr chase, the bulk of the mutant Gag precursor had disappeared, but levels of both cell-associated and extracellular CA proteins were reduced compared to those of wild-type Gag (Fig. 5B, lanes 2). When the intracellular levels of both wild-type and C57A Gag precursor were quantitated over time, mutant Pr55^{Gag} was found to have a half-life of 1.7 hr compared to 2.8 hr for wild-type Gag. This result indicates that mutant C57A exhibits a somewhat higher rate of turnover than that of wild-type Gag.

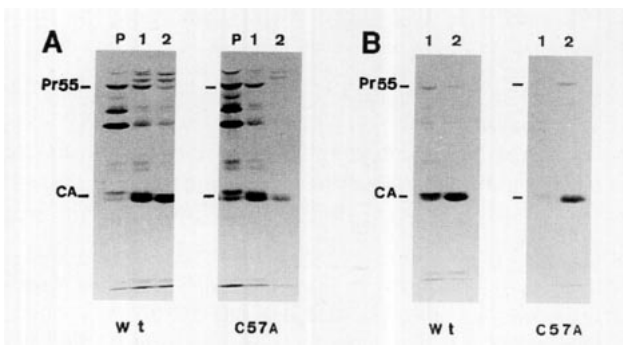

FIG. 5. Kinetics of Gag polyprotein processing and particle release of mutant C57A. (A) Analysis of intracellular viral proteins. CV-1 cells were infected with vv-wtgag (wt) or mutant C57A and pulse-labeled (lane P) for 20 min. After 1.5-hr (lane 1) and 4-hr (lane 2) chases, cells were lysed and virus-specific proteins were immunoprecipitated with pooled sera of infected macaques and analyzed on a SDS–10% polyacrylamide gel. (B) Analysis of particle-associated viral proteins. The culture supernatants of cells infected with vv-wtgag or C57A were collected following 1.5-hr (lane 1) and 4-hr (lane 2) chases. Particles were purified by ultracentrifugation through a sucrose cushion and the presence of viral proteins was analyzed by immunoprecipitation.

In this paper we addressed the role of the three highly conserved cysteine residues of the SIV MA in virus morphogenesis. The SIV MA mutants in which the conserved cysteines were individually replaced (C57A, C83A, C87A) could be arranged into three different phenotypes. Cells expressing mutant C87A synthesized and processed the Gag precursor in a wild-type manner. Furthermore, replacement of cysteine at position 87 appeared to have little effect on particle production and association of Env glycoproteins with mutant particles. By contrast, mutation of C83 caused a major defect in Gag polyprotein processing. This mutation may either induce an altered Gag conformation which conceals the cleavage sites of the polyprotein or prevent PR activation in the context of the Gag–PR polyprotein. In this regard, it has been demonstrated that activation of the viral

PR requires dimerization of the Gag–Pol precursor (*27*); thus, this Gag–PR dimerization may be defective in mutant C83A. Indeed, a linker insertion in the MA domain of HIV-1 disrupted PR activation when an HIV-1 Gag–PR construct was expressed in bacteria (*28*). When particle assembly and release were studied for mutant C83A, a 70% decrease in the level of particle production relative to that of wild-type Gag was observed. Interestingly, mutant C83A-made particles appeared to incorporate Env glycoprotein more efficiently (at least twofold) than the wild-type Gag particles. In support of this evidence, it has been shown that mutations which impair HIV-1 PR activity reduce the levels of particle-associated $Pr55^{Gag}$ compared to those of the wild-type virus (*29*). In addition, virions produced by PR-defective HIV-1 provirus have been found to be more uniformly decorated by Env spikes than the wild-type mature virions (*5*). Substitution of C57 with alanine significantly reduced the ability to produce extracellular particles. When the phenotype of this mutant was investigated in detail by pulse–chase experiments, a moderate increase in the turnover of the Gag polyprotein was observed. This mild effect on protein stability may partly account for the defect in particle production by reducing the levels of Gag proteins competent for assembly. Nevertheless, particle assembly and/or budding may also be affected in mutant C57A. The MA domain of HIV-1 Gag also contains a highly conserved cysteine at position 57. Interestingly, substitution of cysteine 57, as well as deletions including this amino acid, impaired particle production (*15–18*). Therefore, our results, together with those mentioned above, point to a central role of C57 in the process of particle production in both SIV and HIV-1.

A set of double and triple mutants affecting the cysteine residues of SIV MA was also generated and analyzed. The simultaneous replacement of cysteines 83 and 87 resulted in particle production impairment, a phenotype shared with mutant C57A. In mutant C57A/C83A, particle release was also affected but to a lesser extent. By contrast, mutants C57A/C87A and C57A/C83A/C87A produced and released particles with efficiency similar to that of wild-type Gag. It may seem somewhat surprising that mutation of both C57 and C87 as well as the simultaneous replacement of all cysteine residues in the SIV MA appear to have fewer effects on particle assembly than single substitution of C57 or C83. Other workers have experienced a similar situation; it has frequently been observed that double mutants were phenotypically closer to the wild-type molecule than any of the corresponding single mutants (*30–32*). It has been suggested that double mutants may fold into a structure which resembles that of the native molecule, thereby compensating for the effect of the mutations (*33*).

In mutants C57A/C83A, C57A/C87A, and C57A/C83A/C87A, incorporation of the Env glycoprotein into particles was moderately affected; a 40–50% decrease with respect to wild-type Gag particles was observed. In the case of the HIV-1 MA, small in-frame deletions and missense mutations reduced Env glycoprotein incorporation into virions to background levels (*15, 16*). Although the mutations described in these studies were more drastic than those analyzed in our report, the SIV MA may be less sensitive than its HIV-1 counterpart with respect to mutations affecting its interaction with the Env glycoprotein.

The present study, together with our previous report on the polybasic region of the SIV MA (*11*), represent a first stage toward mapping the residues relevant to MA function in SIV morphogenesis.

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